J Pineal Res 1993;14:89–97
Printed in the United States of America—all rights reserved

Journal of Pineal Research
ISSN 0742-3098

ELF magnetic fields, breast cancer, and melatonin: 60 Hz fields block melatonin's oncostatic action on ER^+ breast cancer cell proliferation

Liburdy RP, Sloma TR, Sokolic R, Yaswen P. ELF magnetic fields, breast cancer, and melatonin: 60 Hz fields block melatonin's oncostatic action on ER^+ breast cancer cell proliferation. J. Pineal Res. 1993;14:89–97.

Abstract: In this study we investigated whether a 60 Hz magnetic field can act at the *cellular level* to influence the growth of human estrogen-dependent breast cancer cells. Our experimental design assessed cell proliferation of a human breast cancer cell line, MCF-7, in the absence or the presence of melatonin which inhibits growth at a physiological concentration of 10^{-9} M. In three experiments, continuous exposure to average sinusoidal 60 Hz magnetic fields of 1.90 ± 0.01, 2.40 ± 0.70, and 2.53 ± 0.50 mG, or simultaneous exposure in matched incubators to average 60 Hz magnetic fields of 10.4 ± 2.12, 11.95 ± 2.73, and 11.95 ± 3.28 mG, respectively, had no effect on cell proliferation in the absence of melatonin. When MCF-7 cells were cultured in the presence of 10^{-9} M melatonin, an 18% inhibition of growth was observed for cells in a 2.40 ± 0.70 mG field. This effect was blocked by a 60 Hz magnetic field of 11.95 ± 2.75 mG. In a second experiment, a 27% inhibition of MCF-7 cell growth was observed for cells in a 2.53 ± 0.50 mG magnetic field, and this was blocked by a 60 Hz magnetic field of 11.95 ± 3.28 mG. These results provide the first evidence that ELF frequency magnetic fields can act at the *cellular level* to enhance breast cancer cell proliferation by blocking melatonin's natural oncostatic action. In addition, there appears to be a dose threshold between 2 and 12 mG. The mechanism(s) of action is unknown and may involve modulation of signal transduction events associated with melatonin's regulation of cell growth.

R.P. Liburdy, T.R. Sloma, R. Sokolic, and P. Yaswen

Department of Cell and Molecular Biology, Life Sciences Division, Lawrence Berkeley Laboratory, UC Berkeley, Berkeley, CA 94720

Key words: melatonin—breast cancer—MCF-7 cells—60 Hz magnetic fields

Address reprint requests to Dr. R.P. Liburdy, Bioelectromagnetics Research Facility, Department of Cell and Molecular Biology, Bldg. 74, Lawrence Berkeley Laboratory, Berkeley, CA 94720

Received November 15, 1992;
accepted January 14, 1993.

Introduction

Exposure to ELF magnetic fields are reported to depress or time-shift melatonin secretion in animals [Wilson et al., 1990; Reiter and Richardson, 1990; Lerchl et al., 1991; Spears and Yellon, 1991; Stevens et al., 1992; Reiter, 1992] and such exposures are postulated to be a risk factor in human breast cancer epidemiology [Stevens, 1987; Tynes and Andersen, 1990; Demers et al., 1991; Matanoski et al., 1991]. The underlying mechanism(s) for eliciting such alterations in melatonin secretion *in vivo* are currently receiving much attention, and the body of evidence supports the hypothesis that magnetic fields influence the pineal gland's control over the timed release of melatonin into the circulation. Once released into the circulation, melatonin itself acts at the cellular level and has natural oncostatic activity towards human estrogen-receptor-positive (ER^+) breast cancer cells such as the MCF-7 [Hill and Blask, 1988; Blask, 1990; Cos and Blask, 1990; Cos et al., 1991]. This fact, plus the above findings that magnetic fields influence the release of melatonin into the blood stream, suggests that magnetic fields may play a significant role in breast cancer.

An experiment was designed to address a simple question: Do 60 Hz magnetic fields influence *in vitro* breast cancer cell proliferation, and is the presence of melatonin a modulatory factor in this process? The experimental design employed a well characterized cellular model system, the human breast cancer MCF-7 cell line [Hill and Blask, 1988; Blask, 1990; Cos and Blask, 1990; Cos et al., 1991], to evaluate any direct interaction between the magnetic fields and breast cancer cells, and to determine if melatonin mediated the interaction. A special feature of these *in vitro* experiments is that they relate to interactions at the cellular level which are distal to a magnetic field effect on melatonin release into the blood stream. Thus, the *in vitro* findings presented here complement important *in vivo* studies dealing with ELF magnetic fields and melatonin release into the blood stream. Preliminary results of this research were presented in abstract form [Liburdy et al., 1993b].

Materials and methods

Cells, hormones, and drugs

MCF-7 cells are derived from the pleural effusion of a mammary adenocarcinoma, and, when grown as a monolayer, they possess an epithelial-like morphology [ATCC HTB-22]. Melatonin-sensitive MCF-7 cells at passage #315 were a generous gift of Dr. David Blask of the Mary Imogene Bassett Hospital Research Institute, Cooperstown, NY. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM H-21), supplemented with 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 mg/ml), and L-glutamine (2 mM) (UCSF Cell Culture Facility, San Francisco, CA), at 37°C in a humid atmosphere containing 5% CO_2. Melatonin (N-acetyl-5-methoxytryptamine) was purchased from Sigma Chemical Co. (Product # M-5250 [Lot # 22H7706] St. Louis, MO). Melatonin solution was prepared immediately before use by dissolving crystals in a minimum volume of ethanol and then adding media to achieve the appropriate concentrations.

Exposure systems

Two identical, double-wound, four-coil, Merritt exposure systems were fabricated at the Lawrence Berkeley Laboratory (LBL) for these studies [Merritt et al., 1983; Kirschvink, 1992]. Figure 1a and 1b illustrate the four-coil Merritt design in which there are four double-wound coils wrapped around a square plastic frame (35.5 cm/edge). With the center axis of the coil system used as a reference line and the center point on this axis designating the origin, the four coils had the following spacing with respect to the origin: 16.7 cm, 4.23 cm, −4.23 cm, and −16.7 cm. Commercially available standard speaker cable with two parallel wire tracks was used for the double-wrap cable. Merritt's turns ratio of 26/11/11/26 was followed in winding these four coils (17.0 Ω, 6.57 mH) to optimize a large uniform exposure area in the center volume of the exposure system. Built into the circuit energizing these coils was a switch used to reverse the direction of the current in one of the wires in the speaker cable comprising the double-wound coils. When current is applied in the anti-parallel configuration (passive), the magnetic fields from the double-wound coils cancel, and when current is applied in the parallel configuration (active), a magnetic field is established. This feature enabled a true sham exposure to be performed in one incubator while a second coil system was operated simultaneously for exposure treatment in a second matched incubator. Each coil system was driven by identical signal generators available from Dynascan Corp., Chicago, IL (B&K Precision Model 3020). Each of the four coils comprising the exposure system was shielded by wrapping the wire bundles in two layers of heavy-duty aluminum foil, with a break of several inches, to eliminate the electric field components generated by the wire wrappings.

Field dosimetry was performed using commercially available fluxgate magnetometers. AC magnetic fields were measured using a Hewlett Packard Model 428B fluxgate meter (Cupertino, CA), calibrated by the Magnetic Field Measurements Group at LBL. DC magnetic fields were mapped using the above Hewlett Packard fluxgate meter, and a MAG-01 fluxgate magnetometer from Bartington Instruments, LTD (Oxford, UK). In addition, a FGM-3D1 fluxgate magnetometer from Walker Scientific, Inc. (Worcester, MA) was provided by Dr. Michael Yost of the School of Public Health, UC Berkeley. Active measuring volume of the HP and Bartington probes is a cylindrical volume of approximately 2.0 cm in length and 0.7 cm in diameter. DC field measurements using these three systems agreed to within 2–5%.

The coil systems were placed inside of two identical, water-jacketed Queue incubators (Queue Systems, Inc., Parkersburg, WV, Model 2710) maintained at 37 ± 0.1°C. Identical commercial incubators were used in these studies, since the intensity of the ambient time-varying magnetic field flux density inside of incubators can vary considerably among manufacturers [Berglund et al., 1991;

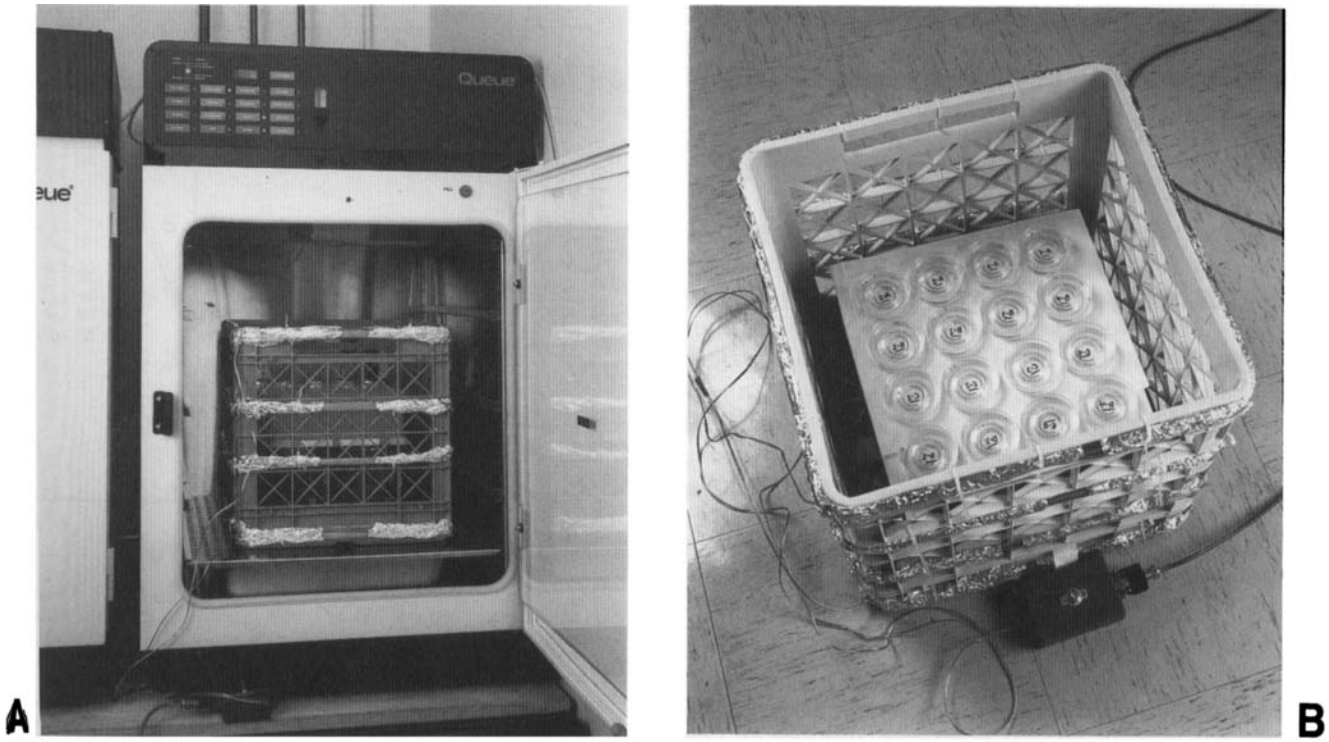

Fig. 1. Double-wound, four-coil Merritt exposure systems. **(A)** View of the exposure coil inside of a Queue Model 2710 incubator as used in these studies. **(B)** View of the exposure coil showing an array of plates positioned in the center plane of the coil. Two plastic frames were wound with double-wrap cable, and were energized in the parallel- or in the antiparallel-configuration so that identical current loads would result in a combined exposure magnetic field (parallel) or in canceled magnetic fields (antiparallel) for the exposure and the control treatment, respectively. Coils were shielded with aluminum foil to eliminate the electric field component. Magnetic field flux density was incident on the surface of the 35 mm tissue culture plates. (See text for details).

Yost and Liburdy, 1992]. When the above coils were operated, either in the active or passive mode, the total current load in the coils was approximately 6.57 mA and this corresponds to an ohmic power dissipation of 734 μW. This did not result in significant heat loading inside the incubators; air temperature in both matched incubators during field operation was maintained to within ±0.1°C, which is the nominal limit of temperature stability for these incubators. Three separate 60 Hz experiments were conducted in the studies presented here, and the inside of each incubator was extensively mapped before and after growth curves were completed. The average (mean ± S.E.) of both measurements for the parallel (active) mode and antiparallel (passive) mode exposures, respectively, corresponded to (a) 10.4 ± 2.12 and 1.90 ± 0.01 mG; (b) 11.95 ± 2.75 and 2.40 ± 0.70 mG; (c) 11.95 ± 3.28 and 2.53 ± 0.05 mG. The Merritt coils used in these studies generate very uniform field values (±2%) over the central area where cells were placed [Kirschvink, 1992]. The variations about mean values observed for these 60 Hz magnetic fields are attributed to the ambient background 60 Hz magnetic fields present in the laboratory during the seven month period in which these studies were conducted. DC fields were mapped at the same positions that 60 Hz fields were measured and values in incubator 1 and 2 were 142 ± 33 mG and 135 ± 6 mG, respectively. Plate positions in the exposure volume were systematically randomized during exposures, with matched active vs. passive exposures conducted simultaneously. Randomization of plates followed a pattern in which plates were rotated through exposure positions within the central volume of the coil, which was within the ±2% isocontour boundary of the Merritt coils [Kirschvink, 1992] on days during cell counting and feeding. Coils were left energized during the entire period of cell growth in these studies; thus, culture plates were removed for feeding and for the addition of melatonin with the fields continuously present. Cells were first placed in the energized active or passive coils after cell attachment (see below) when

melatonin was added. Time for feeding was kept to a minimum, and, on average, plates were out of the fields during feeding for approximately 5–10 minutes.

Culture techniques

MCF-7 cells were initially grown in 60 mm plates or T-75 flasks prior to experiments. Cells employed in these studies correspond to passage #318, 319, and 320. Cells were harvested in 0.2% EDTA phosphate buffer (2 g/L Na_2-EDTA, 8 g/L NaCl, 0.2 g/L KH_2PO_4, 1.15 g/L Na_2HPO_4), or with trypsin solution (0.50 g/L Trypsin, 0.5 g/L EDTA, 1.0 g/L glucose, and 0.58 g/L $NaHCO_3$) from the seeding vessel and diluted in DMEM. Cells were employed at seeding densities between $0.1–0.2 \times 10^5$ cells/35 mm culture dish in two ml of media. After cells became firmly attached, four hours after seeding, the plating medium was replaced with freshly prepared, melatonin-enriched medium (10^{-9} M) or DMEM alone. On alternate days, cells were harvested from plates in each treatment group by incubation with one ml of trypsin solution at 37°C for two min and counted using a hemocytometer; cell growth was expressed as cell/ml which is equivalent to cells/plate in these studies. Triplicate plates were used for each counting day. Fresh medium, with or without melatonin, as required, was fed to the remaining plates twice weekly. In these studies no gross morphological differences as revealed by transmitted light microscopy were noticed for MCF-7 cells grown in background compared to applied 60 Hz magnetic fields.

Statistical analysis

All data were tested for statistical significance using the multifactor analysis of variance program in Statgraphics (Manugistics, Inc., Rockville, MD, USA).

Results

Comparison of MCF-7 cell growth in matched incubators

We determined whether MCF-7 cells would exhibit identical growth curves in matched incubators in the presence of ambient background 60 Hz magnetic fields. The background magnetic field in the incubators was mapped and was found to fall within the range 1.9 ± 0.01 mG to 2.53 ± 0.50 mG. Field mapping in the laboratory room, as well as inside the incubators, indicated that the 60 Hz background field was similar throughout both incubators when unenergized or energized in the antiparallel mode. For these growth curves, cells at passage #319 were

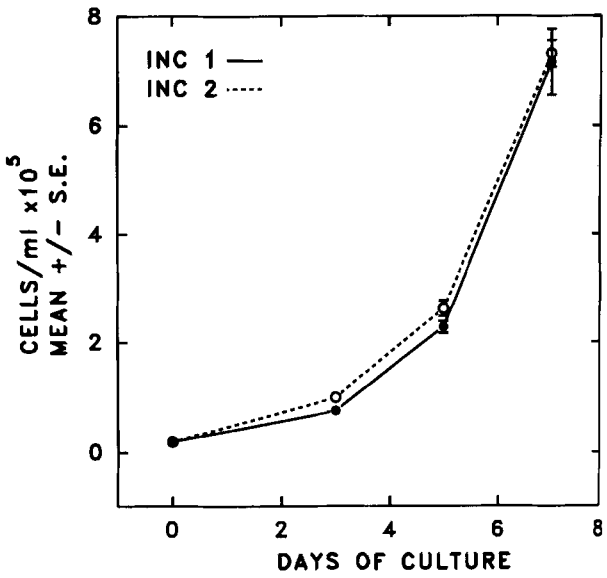

Fig. 2. Comparison of MCF-7 cell growth in matched incubators. Growth curves for MCF-7 cells from passage #319 for cells in matched Model 2710 Queue incubators 1 and 2. Cells were seeded at 0.15×10^5/plate and cell proliferation was followed by hemocytometer count over seven days. Cells were positioned inside of the exposure coils with the coils unenergized (see text).

placed inside of the two incubators with the Merritt coils in place but unenergized, with the cells in the same position as used for exposure treatment. Figure 2 depicts growth curves for MCF-7 cells during simultaneous cell proliferation assays in incubators 1 (solid line) and 2 (dashed line). MCF-7 cells were seeded to attain exponential growth and to be 60–80% confluent by day 7; cell growth was observed to be identical in the matched incubators. This indicates that MCF-7 cells grow identically in the presence of ambient background magnetic fields in these two matched incubators.

60 Hz magnetic fields do not influence MCF-7 cell growth

Next we addressed the question of whether 60 Hz magnetic fields influence normal growth of MCF-7 cells in the absence of melatonin. Simultaneous cell proliferation assays were performed as described above for Figure 2, but with the coils in the matched incubators energized in either the antiparallel (passive) or in the parallel (active) configuration. Energization in the antiparallel configuration, which cancels the applied magnetic field, resulted in a similar magnitude ambient background magnetic field as those depicted in Figure 2. Three different growth curve experiments were conducted on different days with MCF-7 cells from different passages. Figures 3a, 3b, and 3c show typical expo-

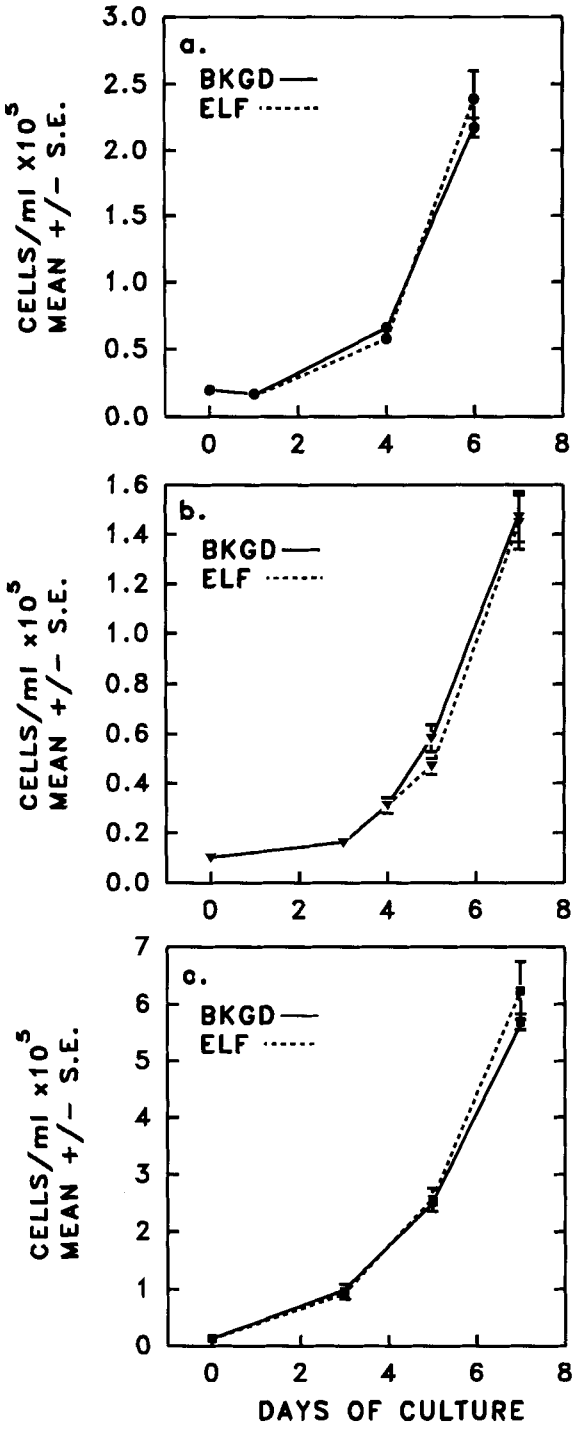

Fig. 3. 60 Hz magnetic fields do not influence MCF-7 cell growth (no melatonin). Cells from three different passages were characterized for cell proliferation in the absence of melatonin in three different experiments. Growth curves were performed as in Figure 2. Incubator 1 was used for 60 Hz (ELF) exposures and Incubator 2 was used for background (BKGD) 60 Hz field treatments. **(a)** Cells from passage #318 seeded at 0.3×10^5/plate. BKGD: 1.90 ± 0.01 mG, ELF: 10.4 ± 2.12 mG. **(b)** Cells from passage #319 seeded at 0.15×10^5/plate. BKGD: 2.40 ± 0.70 mG, ELF: 11.95 ± 2.75 mG. **(c)** Cells from passage #320 seeded at 0.137×10^5/plate. BKGD: 2.53 ± 0.50 mG, ELF: 11.95 ± 3.28 mG.

nential growth curves for MCF-7 cells in the presence of the background or the applied magnetic field for cells at passage #318, #319, and #320, respectively. Background (BKGD) and applied magnetic fields were: (3a) 1.90 ± 0.01 mG and 10.4 ± 2.12 mG; (3b) 2.40 ± 0.70 mG and 11.95 ± 2.75 mG; and (3c) 2.53 ± 0.50 mG and 11.95 ± 3.28 mG. Comparison of the growth curves from these three experiments reveals that growth of MCF-7 cells is typically exponential and similar in the presence of the background 60 Hz field compared to that for the applied magnetic field. These data support the finding that cell proliferation of the MCF-7 cells employed here is not influenced by magnetic fields of 1.90–2.53 mG or 10.4–11.95 mG.

60 Hz magnetic fields block melatonin's natural oncostatic action on MCF-7 cell growth

Melatonin is oncostatic and inhibits the proliferation of MCF-7 cells when present in cell culture media at concentrations corresponding to the physiological range of 10^{-9} to 10^{-11}M [Cos and Blask, 1990]. We performed experiments to confirm melatonin's inhibition of MCF-7 cell growth. Growth curves for MCF-7 cells of passage #319 in the absence or in the presence of 10^{-9} M melatonin are depicted in Figure 4a. In these experiments, cells were placed in the incubator with the exposure coils energized in the antiparallel configuration (passive) so that a background 60 Hz magnetic field of 2.40 ± 0.70 mG was present. Cell proliferation revealed typical exponential growth over the 7-day period. When melatonin was present, an 18% inhibition of growth was detected at day 7; this inhibition was statistically significant ($P = 0.017$).

We note that MCF-7 cells display heterogeneity in their response to melatonin, and that some subclones can exhibit growth inhibition of up to 70% in the presence of 10^{-9} M melatonin between days 5–7 [Hill and Blask, 1988; Cos and Blask, 1990]. There is also evidence that the melatonin response is serum-dependent, which suggests that a factor(s) in serum is permissive for melatonin inhibition of MCF-7 cell growth. In addition, melatonin-sensitive MCF-7 subclones may exhibit different sensitivity to melatonin as passage number increases; this may reflect the fact that, as passage number increases, MCF-7 cells generally exhibit shorter doubling times with altered growth properties. Also, some MCF-7 subclones may exhibit no sensitivity to melatonin—this may depend on passage number, serum factors, or other unknown parameters. Sub-clone sensitivity of human cancer cell lines, such as the MCF-7 cell, to melatonin has

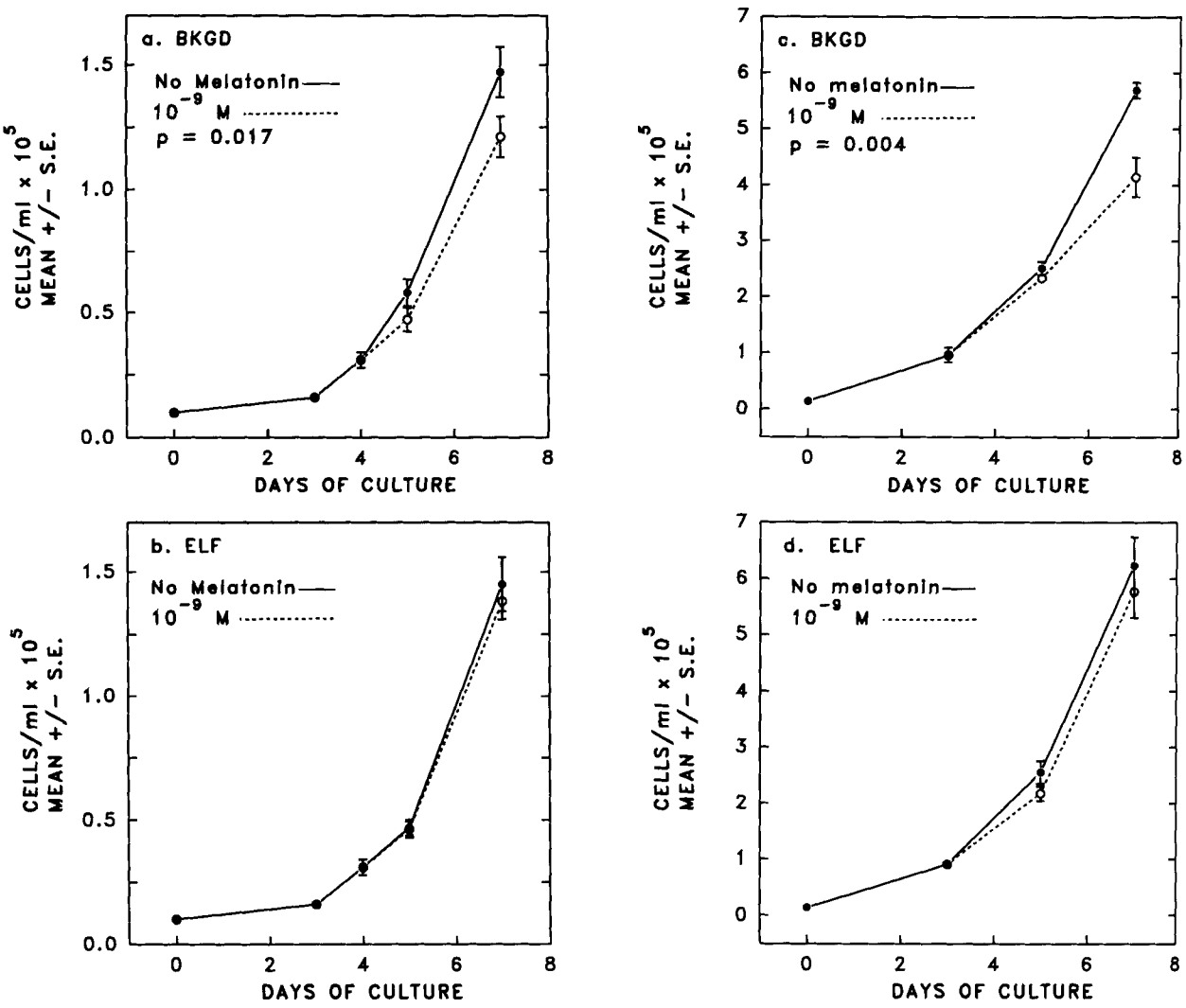

Fig. 4. 60 Hz magnetic fields block melatonin's action on MCF-7 cell growth. Cells from passage #319 were seeded at 0.15×10^5/plate and grown in media containing either no melatonin or 10^{-9} M melatonin, in an incubator with a background (BKGD) 60 Hz magnetic field of 2.40 ± 0.70 mG, or in an incubator with a 60 Hz (ELF) magnetic field of 11.95 ± 2.75 mG. **(a)** Melatonin exhibits an 18% inhibition in MCF-7 cell proliferation at day 7 ($P = 0.017$), and **(b)** ELF magnetic fields block melatonin's growth inhibition of MCF-7 cells. Cells from passage #320 were seeded at 0.137×10^5/plate and grown in media containing either no melatonin or 10^{-9} M melatonin in an incubator with a background (BKGD) 60 Hz magnetic field of 2.53 ± 0.50 mG, or in an incubator with a 60 Hz (ELF) magnetic field of 11.95 ± 3.28 mG. **(c)** Melatonin exhibits an 27% inhibition in MCF-7 cell proliferation at day 7 ($p = 0.004$), and **(d)** ELF magnetic fields block melatonin's growth inhibition of MCF-7 cells.

been noted by L'Hermite-Baleriaux and de Launoit [1992]. In all of the studies presented here, we employed MCF-7 cells that were responsive to melatonin, we have also identified our source of cells, as well as the passage number, the source of serum, and the media we used.

To address the question of whether an applied 60 Hz magnetic field can influence melatonin's natural oncostatic action, cells from the same passage #319 were placed in the matched incubator in the absence or presence of 10^{-9} M melatonin plus an applied 60 Hz magnetic field of 11.95 ± 2.75 mG, and cell growth was followed. These cell growth curves were obtained with the exposure coils energized in the parallel configuration (active). Figure 4b shows an exponential growth curve for the MCF-7 cells in absence of melatonin (solid line) that is superimposable with that obtained in the background field (compare with Figure 4a). This is consistent with results shown in Figure 3, in which magnetic fields alone do not alter MCF-7 cell growth. However, Figure 4b reveals that the 11.95 magnetic field in

the presence of melatonin at 10^{-9} M completely blocked melatonin's growth inhibition.

The above series of experiments was repeated using the same exposure coils, incubators, exposure conditions, and melatonin concentration (10^{-9} M), but with cells at passage #320. Figure 4c depicts MCF-7 cell growth in the absence or presence of melatonin in a background field of 2.53 ± 0.50 mG. Exponential growth was observed in the absence of melatonin. When melatonin was present a growth inhibition of 27% was observed at day seven that was statistically significant ($P = 0.004$). Simultaneously, in the matched incubator, MCF-7 cells were characterized for cell proliferation in the absence or presence of melatonin plus an applied ELF magnetic field of 11.95 ± 3.28 mG. These growth curves are presented in Figure 4d. Typical exponential growth was observed for cells in the absence of melatonin. However, when melatonin was present, the 60 Hz magnetic field was observed to block completely melatonin's growth inhibition.

In both of the experiments presented in Figure 4, the seeding density, melatonin, and serum were essentially equivalent, but the passage number was different (319 vs. 320). Cells at passage 319 displayed slower growth than cells from passage 320. The slower growth of cells at passage 319 may have contributed to an observed 18% growth inhibition of melatonin, compared to a 27% inhibition of growth for cells at passage 320.

Discussion

The findings presented here indicate that 60 Hz magnetic fields of 11.95 mG block melatonin's growth inhibition of MCF-7 breast cancer cells when melatonin is present at a physiological concentration of 10^{-9} M; 2.40 to 2.53 mG fields did not block melatonin activity (Figure 4). In the absence of melatonin, magnetic fields had no effect on MCF-7 cell growth (Figures 3 and 4). The above findings taken together indicate that (a) this *in vitro* effect occurs at the cellular level, (b) it involves an interaction that requires the presence of melatonin, and (c) a dose threshold appears to exist between 2 and 12 mG. These findings represent the first evidence for a *cellular level* response to ELF magnetic fields that is dependent on melatonin.

Several models have been proposed to elucidate the link between ELF magnetic field exposure and breast cancer incidence [Wilson et al., 1990; Reiter and Richardson, 1990; Reiter, 1992; Stevens et al., 1992]. These models suggest that the most important aspect of this link is a decrease in melatonin secretion in response to an *in vivo* magnetic field exposure concomitant with enhancement in the production of prolactin and estrogen; the latter events are thought to increase the growth of susceptible breast epithelial cells [Cohen et al., 1978]. The study presented here deals with *in vitro* exposure of breast cancer cells and, therefore, relates to a subset of cellular events that occur distal to an *in vivo* effect on the pineal gland which regulates melatonin's secretion into the blood stream. The results reported here raise the possibility that a direct interaction between ELF magnetic fields, breast cancer cells, and melatonin may exist at the *cellular level*. These findings, therefore, complement important existing *in vivo* research [Wilson et al., 1990; Reiter and Richardson, 1990; Reiter, 1992; Stevens et al., 1992].

These cellular interactions most likely involve receptor activation and signal transduction pathways in the MCF-7 cell. In the tissue culture media employed in these studies, the relevant mitogen which triggers ER^+-MCF-7 cell proliferation is estrogen. We confirmed the findings of Blask and colleagues that melatonin inhibits estrogen-dependent growth of MCF-7 cells [Cos and Blask, 1990]. Our finding that an ELF magnetic field blocks melatonin's action may be most simply explained by the magnetic field interfering with melatonin interaction with MCF-7 cells at a receptor binding site. It would be of interest to conduct binding studies using a compound that interferes directly with melatonin binding to MCF-7 cells, however a receptor for melatonin in the MCF-7 cell has not yet been identified. Melatonin has been postulated to exert its antiproliferative effect on human breast cancer cells through estrogen-dependent mechanisms as well as estrogen-independent mechanisms, based on melatonin's ability to block estradiol's mitogenic effect in MCF-7 cells in monolayer culture, and on estradiol's partial ability to rescue MCF-7 cells from melatonin's growth inhibition [Cos et al., 1991]. Thus, melatonin may interact with a variety of receptors in MCF-7 cells. Future studies should consider the possibility of estrogen receptor involvement.

There is experimental evidence for ELF magnetic fields altering signal transduction events. In *in vitro studies with transformed HL60 cells, transcription* and translation are altered in cell culture during exposure to ELF magnetic fields [Goodman and Shirley-Henderson, 1991]. Experimental evidence now exists for a 60 Hz field effect on one of the earliest signal transduction markers, $[Ca^{2+}]_i$, and was first reported for mitogen-activated thymic lymphocytes [Liburdy, 1992]. Alterations in $[Ca^{2+}]_i$ were not observed during 60 Hz field

exposure of resting cells, but approximately 100 seconds after addition of the lymphocyte mitogen Con-A, changes in $[Ca^{2+}]_i$ were detected. Alterations of an early ST marker like $[Ca^{2+}]_i$ are expected to occur at the cell surface and propagate down the signal transduction cascade to influence expression of such proto-oncogenes as c-MYC [Liburdy et al., 1993a]; this is a plausible biological mechanism that links ELF magnetic fields with the cell surface to subsequent gene expression events such as DNA synthesis and cell proliferation [Liburdy, 1992; Yost and Liburdy, 1992; Liburdy et al., 1993a]. To provide support for this interaction pathway, we have recently observed ELF-increased $[Ca^{2+}]_i$ and ELF-increased c-MYC mRNA induction in the same thymic lymphocytes [Liburdy, et al., 1993a]. The latter finding is consistent with c-MYC mRNA induction reported by Goodman and Henderson [1991] in HL60 cells. In addition, interesting studies by Luben [1993] indicate that pulsed 72 Hz magnetic fields are capable of inhibiting the activation of cAMP accumulation by β-adrenergic agonists in pineal cell cultures and in osteoblasts. They interpreted their findings to suggest that G-protein linked receptors on the cell surface are influenced by the fields, perhaps through small changes in the charge distribution of the cell membrane. The general feature that cell surface receptors involved in signal transduction are influenced by magnetic fields is consistent with the above lymphocyte studies (the T-cell receptor is G-protein linked) and the pineal gland studies (the β-adrenergic receptor is G-protein linked). Given these interactions and the findings presented above, we hypothesize that a mechanism for magnetic field action on MCF-7 growth may involve signal transduction events that (a) interfere with melatonin interaction with MCF-7 cells at the receptor level to alter subsequent signal transduction sequelae, and/or (b) activate other mitogen receptors, in the presence of melatonin, to overcome melatonin's anti-mitogenic action. The estrogen receptor is a possible candidate for the latter interaction, as discussed above, and future studies should address such issues.

Corollary issues that should also be investigated in future studies are (a) the possible role that serum factors may play in mediating melatonin-sensitive cell growth of MCF-7 cells in 60 Hz magnetic fields, and, (b) the effect of 60 Hz magnetic fields on MCF-7 cell growth at different melatonin concentrations. Since this study was conducted at a physiological dose of 10^{-9} M melatonin, studies are in progress over the concentration range corresponding to pharmacological (10^{-7} M) through sub-physiological (10^{-11} M) doses of melatonin. This should yield important information concerning whether the blocking effect of 60 Hz magnetic fields on melatonin-sensitive MCF-7 cell growth represents an absolute decrease in melatonin activity or a shift of the melatonin dose response curve.

Acknowledgments

The helpful discussions and encouragement of Drs. Bary Wilson, James Morris, David Blask, and Russ Reiter in these studies are most gratefully acknowledged. We thank M. Wiesendanger for technical assistance in these studies. Research was supported in part by the Office of Energy Management, Utilities System Division, and the Office of Health and Environmental Research, of the Department of Energy, under contract DE-AC03-76SF00098.

Literature Cited

BERGLUND, A., H. K. MILD, M. SANDSTROM, M.-O. MATTSSON (1991) Background ELF magnetic fields in incubators. Presented at the 13th Annual Meeting of the Bioelectromagnetics Society, Salt Lake City, UT. June 23–27.

BLASK, D.E. (1990) The emerging role of the pineal gland and melatonin in oncogenesis. In: Extremely Low Frequency Electromagnetic Fields: The Question of Cancer. B.W. Wilson, R.G. Stevens, L.E. Anderson, eds. Battelle Press, Columbus, OH, pp 319–325.

COHEN, M., M. LIPPMAN, B. CHABNER (1978) Role of pineal gland in etiology and treatment of breast cancer. Lancet 2:814–816.

COS, S., D.E. BLASK (1990) Effects of the pineal hormone melatonin in the anchorage-independent growth of human breast cancer cells (MCF-7) in a clonogenic culture system. Cancer Lett. 50:115–119.

COS, S., D.E. BLASK, A. LEMUS-WILSON, A.B. HILL (1991) Effects of melatonin on the cell cycle kinetics and estrogen-rescue of MCF-7 human breast cancer cells in culture. J. Pineal Res. 10:36–42.

DEMERS, P.A., D.B. THOMAS, K.A. ROSENBLATT, L.M. JIMENEZ, A. MCTIERNAN, H. STALSBERG, A. STEMHAGEN, W.D. THOMPSON, M.G. MCCREA CURNEN, W. SATARIANO, D.F. AUSTIN, P. ISACSON, R.S. GREENBERG, C. KEY, L.N. KOLONEL, D.W. WEST (1991) Occupational exposure to electromagnetic fields and breast cancer in men. Am. J. Epidemiol. 132:775–776.

GOODMAN R., A. SHIRLEY-HENDERSON (1991) Transcription and translation in cells exposed to extremely low frequency electromagnetic fields. Bioelectrochem. Bioenerg. 25:335–355.

HILL, S.M., D.E. BLASK (1988) Effects of pineal hormone melatonin on the proliferation and morphological characteristics of human breast cancer cells (MCF-7) in culture. Cancer Res. 48:6121–6126.

KIRSCHVINK, J.L. (1992) Uniform magnetic fields and double-wrapped coil systems: Improved techniques for the design of bioelectromagnetic experiments. Bioelectromagnetics 13: 401–411.

LERCHL, A., K.O. HONAKA, R.J. REITER (1991) Pineal gland

magnetosensitivity to static magnetic fields is a consequence of induced electric currents (eddy currents). J. Pineal Res. 10:108–116.

L'HERMITE-BALERIAUX, M., Y. DE LAUNOIT (1992) Is melatonin really an in vitro inhibitor of human breast cancer cell proliferation? In Vitro Cell. Dev. Biol. 28A:583–584.

LIBURDY R.P. (1992) Calcium signaling in lymphocytes and ELF fields: Evidence for an electric field metric and a site of interaction involving the calcium ion channel. FEBS Lett. 301:53–59.

LIBURDY, R.P., D.E. CALLAHAN, T.R. SLOMA, P. YASWEN (1993a) Intracellular calcium, calcium transport, and c-MYC mRNA induction in lymphocytes exposed to 60 Hz magnetic fields: The cell membrane and the signal transduction pathway. Symposium of the First World Congress for Electricity and Magnetism in Biology & Medicine, Lake Buena Vista, FL, June 14–19, 1992. In press.

LIBURDY, R.P., M. WEISENDANGER, R. SOKOLIC, P. YASWEN (1993b) ELF magnetic fields and melatonin-induced growth inhibition of ER^+ breast cancer cells. Symposium of the First World Congress for Electricity and Magnetism in Biology & Medicine, Lake Buena Vista, FL, June 14–19, 1992. In press.

LUBEN, R.A. (1993) Effects of low energy electromagnetic fields (EMF) on signal transduction by G-protein linked receptors. Symposium of the First World Congress for Electricity and Magnetism in Biology & Medicine, Lake Buena Vista, FL, June 14–19, 1992. In press.

MATANOSKI, G.M., P.N. BREYESE, E.A. ELLIOT (1991) Electromagnetic field exposure and male breast cancer. Lancet 33:737.

MERRITT, R., C. PURCELL, G. STROINK (1983) Uniform magnetic field produced by three, four, and five square coils. Rev. Sci. Instrum. 54:879–882.

REITER, R.J. (1992) Changes in circadian melatonin synthesis in the pineal gland of animals exposed to extremely low frequency electromagnetic radiation: A summary of observations and speculation on their implications. In: M.C. Moore-Ede, S.S. Campbell, R.J. Reiter, eds. Electromagnetic Fields and Circadian Rhythmicity. Birkhauser, Boston, pp. 13–17.

REITER, R.J., B.A. RICHARDSON (1990) Magnetic field effects on pineal indoleamine metabolism and possible biological consequences. FASEB J. 6:2283–2287.

SPEARS, L.C., S.M. YELLON (1991) The effect of acute exposure to 60 Hz EMF on the nocturnal melatonin rhythm in the adult Djungarian hamster. Presented at the 13th Annual Meeting of the Bioelectromagnetics Society, Salt Lake City, UT, June 23–27.

STEVENS, R. (1987) Electric power use and breast cancer: A hypothesis. Am. J. Epidemiol. 125:556–561.

STEVENS, R.G., S. DAVIS, D.B. THOMAS, L.E. ANDERSON, B.W. WILSON (1992) Electric power, pineal function, and the risk of breast cancer. FASEB J. 6:853–860.

TYNES, T., A. ANDERSEN (1990) Electromagnetic fields and male breast cancer. Lancet 336:1596.

WILSON, B.W., C.W. WRIGHT, J.E. MORRIS, R.L., BUSCHBOM, D.P. BROWN, D.L. MILLER, R. SOMMERS-FLANNIGAN, L.E. ANDERSON (1990) Evidence for an effect on ELF electromagnetic fields on human pineal gland function. J. Pineal Res. 9:259–269.

Yost, M.G., R.P. Liburdy (1992) Time-varying and static magnetic fields act in combination to alter signal transduction in the lymphocyte. FEBS Lett. 296:117–122.